PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : G01N 33/569, C07K 7/04 A1

(11) International Publication Number: WO 91/13360

(43) International Publication Date: 5 September 1991 (05.09.91)

(21) International Application Number: PCT/SE91/00114

(22) International Filing Date: 18 February 1991 (18.02.91)

(30) Priority data:
9000595-0 20 February 1990 (20.02.90) SE

(71) Applicant *(for all designated States except US)*: REPLICO MEDICAL AKTIEBOLAG [SE/SE]; Storgatan 5, S-234 00 Lomma (SE).

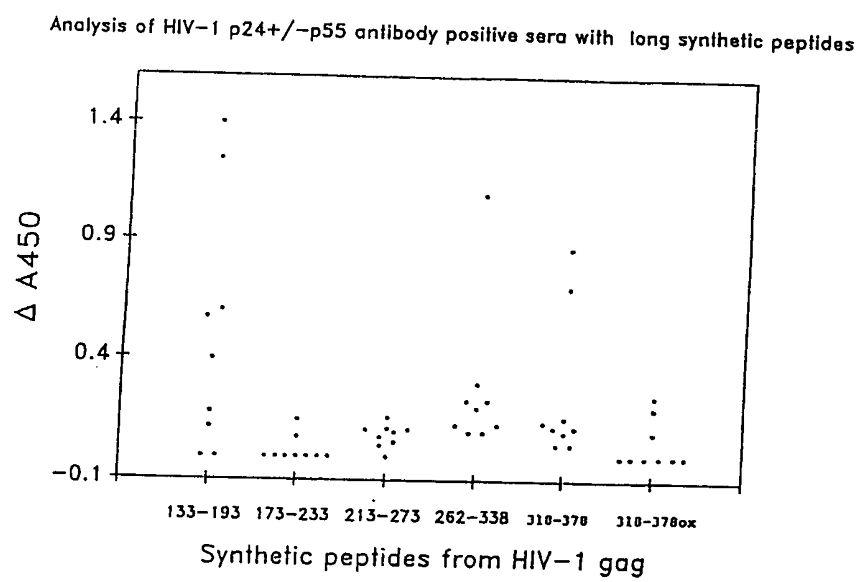
(72) Inventors; and
(75) Inventors/Applicants *(for US only)* : BLOMBERG, Jonas [SE/SE]; Storgatan 5, S-234 00 Lomma (SE). PIPKORN, Rüdiger [SE/SE]; Ugglumsvägen 12, S-433 62 Partille (SE).

(74) Agent: AWAPATENT AB; Box 5117, S-200 71 Malmö (SE).

(81) Designated States: AT, AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH, CH (European patent), CM (OAPI patent), DE, DE (European patent), DK, DK (European patent), ES, ES (European patent), FI, FR (European patent), GA (OAPI patent), GB, GB (European patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL, NL (European patent), NO, PL, RO, SD, SE, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent), US.

Published
With international search report.

(54) Title: NEW HIV p24 PEPTIDES, DIAGNOSTIC ANTIGENS AND DISCRIMINATIVE IMMUNOASSAY METHOD



(57) Abstract

Peptides or antigenic parts thereof and a diagnostic antigen are described, which antigen has the ability of binding to antibodies which have a binding affinity for compounds containing an amino acid sequence corresponding to an epitope or clusters of epitopes of HIV-1 p24, wherein it mainly consists of said antigen, which is selected from said peptides or antigenic parts thereof. Further, a method of discriminating between a false and true diagnosed HIV-positive serum sample from a subject using an immunoassay, wherein said diagnostic antigen is used, is described.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MN	Mongolia
BE	Belgium	GA	Gabon	MR	Mauritania
BF	Burkina Faso	GB	United Kingdom	MW	Malawi
BG	Bulgaria	GN	Guinea	NL	Netherlands
BJ	Benin	GR	Greece	NO	Norway
BR	Brazil	HU	Hungary	PL	Poland
CA	Canada	IT	Italy	RO	Romania
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland	KR	Republic of Korea	SN	Senegal
CI	Côte d'Ivoire	LI	Liechtenstein	SU	Soviet Union
CM	Cameroon	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
DE	Germany	MC	Monaco	US	United States of America
DK	Denmark				

New HIV p 24 peptides, diagnostic antigens and discriminative immunoassay method.

The present invention relates to new peptides, to a diagnostic antigen having the ability of binding to antibodies which have a binding affinity for compounds containing an amino acid sequence corresponding to an epitope or a cluster of epitopes of HIV-1 p24 and to a method of discriminating between a false and true diagnosed HIV-1 p24 protein antibody positive serum sample using an immunoassay.

Background art

For diagnosis of HIV-1 infection of individuals a standard HIV-1 antibody screening test, usually based on an enzyme immunoassay (EIA), is initially performed with a serum sample from the subject. If a certain cut off value is exceeded in the test result, the sample is considered positive, i.e. the subject is probably infected with HIV-1. For confirmation of this preliminary diagnosis, confirmatory tests are performed, because the serum sample might be positive even though the subject is not HIV-1 infected.

The reasons for a positive result in the tests could be the following:

a) HIV-1 antibodies are present, i.e. the individual is HIV-1 infected (true HIV-1 seropositivity)

b) HIV antibodies are present, but the person is not HIV infected

c) antibodies against contaminating components, for example cellular proteins, are present if the virus used for the assay has been produced by eukaryotic cells, or bacterial proteins if the protein used for the assay is a recombinant protein produced by bacteria

d) the test has technical deficiencies, which lead to a positive reaction, for example insufficient blocking, bacterial growth in buffers etc.

It has been observed that a small portion of sera from humans unlikely to be HIV-1 infected contains antibodies against one or a few HIV-1 proteins, mostly derived from the interior gag part of the virus. One example of such a protein is the p24 protein. Such reactivities can give rise to "indeterminate" HIV-1 electroforetic immunoblot (EIB) reactions. They are a constant cause of concern in laboratories responsible for confirmation of HIV-1 antibody screening results. Anti-p24 reactions is a major diagnostic problem in both developed and developing nations. It is costly to run several confirmatory tests on each of these sera. The elucidation of the biological reason for these reactions and the development of tests facilitating the discrimination of "true" from "false" HIV-1 antibodies then are important objects for research.

One of the most common types of false-positive HIV-1 EIB reactivity is characterised by a reactivity with p24 protein with or without its precursor p55 in the gag part, but with no other HIV-1 protein. Most likely a false HIV-1 antibody test is obtained when tests based on whole virus are used, and in particular all tests in which p24 proteins are involved. A single anti-p24 band can be a signal of an early HIV-infection, be due to an HIV-2-infection, be the consequence of a "cryptic" anti-HIV serological reaction (see Ranki AM, Krohn M, Allain JP, et al.: Long latency precedes overt seroconversion in sexually transmitted human immunodeficiency virus infection. Lancet 1987, ii:589-593), or be a false-positive reaction in a person who never encountered HIV.

The most common confirmatory tests are Western blotting, inhibition EIA, peptide EIA and radioimmuno precipitation. Usually 3 or 4 confirmatory tests are necessary to discriminate between false and true positive sera.

In Western blotting purified whole HIV-1 virus is fractionated by molecular weight using electroforesis on a polyacrylamide gel. The separated HIV-1 proteins, which function as viral antigens, are then transferred from the

gel via electrophoretic blotting to a nitrocellulose membrane. Alternatively, recombinant proteins or synthetic peptides corresponding to HIV-1 proteins can be used as antigens. The blot is cut into strips which are then reacted with test and control serum or plasma samples. During incubation, if anti-HIV-1 antibodies are present in the sample, they will bind to the viral antigens on the nitrocellulose strips. This antigen-antibody complex is detected by an antihuman immunoglobulin G (IgG) antibody conjugated to an enzyme, that in presence of a substrate will produce a colored band on the strips. If a full (normal) HIV-1 seropositivity according to a) above exists, antibodies against almost all of the virus proteins are present. This test is rather sensitive and it permits separation of the immune response to different proteins originating from different parts of a virus organism.

Reactions against both <u>gag</u> (<u>gag</u> = p24, p17, p55) proteins from the interior parts of the virus and <u>env</u> (<u>env</u> = gp41, gp160, gp120) proteins from the envelope of the virus are required for this test. The risk for such a pattern to arise randomly is very small. If a patient has antibodies against a few single virus proteins, the unreliability is more pronounced. Then it is necessary to distinguish an incomplete but true immune response against HIV-1 from an antibody reaction against another protein with ability to induce the same antibodies as a HIV-1 protein (false positivity). Reactions with p17, p24 and p55 are most common of the <u>gag</u> reactions, wherein p17 reactions are most common and p55 are least common. The appearance and the intensity of the different bands in EIB are dependent on if the infection is recent. Consequently, it is impossible to be absolutely sure if a single <u>gag</u> band is true or false using only a Western blot. This is a considerable disadvantage of the method.

Inhibition EIA (competition EIA) is a technique that discriminates between false and true reactivity by competition of labelled true positive antibodies and the serum

sample from the patient for binding to HIV-1 bound to a solid phase. If the patient serum sample inhibits the labelled antibodies it is clear that they bind to the same sites on the HIV-1 proteins and that the antibodies in the patient serum sample bind as strong as the probed antibodies. If the reaction is false, none of those conditions are fullfilled. However, as this test is a negative test, i.e. it is based on non-reactivity of possibly false positive antibodies and not on direct indication, it is associated with a certain unreliability.

Peptide EIA is a technique that further separates the immune response to single epitopes. By this technique it is possible to discriminate between true and false reactions because the reasons for the immune response are different. It is possible to use peptide EIA to indicate that antibodies raised against env proteins are present. Peptide EIA is a more sensitive technique than Western blot in indicating env antibodies. However, at present the use of this technique is somewhat restricted because the test may fail if there are slight variations in peptide sequence of the virus like in certain patients from Africa.

In the radioimmunoprecipitation test, RIPA, the antibodies from the patient serum sample are bound to radioactively labelled HIV-1 proteins. The antigen-antibody complexes are immobilised on protein A beads, and are then solubilised and subjected to electrophoresis in polyacrylamide. This test requires high affinity of the antibodies and it is rather sensitive. It can discriminate fairly well between true and false reactions. It is particularly advantageous to study env reactivity by RIPA. The disadvantages with this test is that it is rather expensive, time consuming and laborious. Further, there is an aspect of hazard in working with radioactive material.

Consequently there is a great demand for a method of reliable, fast, simple and cheap discrimination between false and true HIV-1 reactions, especially gag reactions,

for example anti-p24 reactions. This is of great importance, especially for developing countries, where false seropositivity can be a large problem.

This demand is satisfied by the present invention. Compared to the known technique the method according to the present invention is simpler for discriminating between true and false HIV-1 anti-p24 reactions. Furthermore, it is safer and in certain respects more reliable than known techniques. It is also fast, and the test is performed in 3-4 h, compared to known techniques, in which up to 24 h are necessary. Finally, it should be at least ten times cheaper per test than a Western blot and much cheaper than the other conventional confirmatory tests. In combination with the conventional methods, the method gives an additional safety margin, because it positively identifies the false positive reactions. To sum up, it is possible to select additional not HIV-1 infected individuals from a group of individuals which have been diagnosed as possibly HIV-1 positive, compared to any hitherto known method.

Description of the invention

In one aspect the present invention relates to peptides of the formula

a) H-X-Pro-Ile-Val-Gln-Asn-Ile-Gln-Gly-Gln-Met-Val-His-
-Gln-Ala-Ile-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys-
-Val-Val-Glu-Glu-Lys-Ala-Phe-Ser-Pro-Glu-Val-Ile-Pro-
-Met-Phe-Ser-Ala-Leu-Ser-Glu-Gly-Ala-Thr-Pro-Gln-Asp-
-Leu-Asn-Thr-Met-Leu-Asn-Thr-Val-Gly-Gly-X-Z

b) H-X-Val-His-Gln-Ala-Ile-Ser-Pro-Arg-Thr-Leu-Asn-Ala-
-Trp-Val-X-Z

c) H-X-Ser-Ala-Leu-Ser-Glu-Gly-Ala-Thr-Pro-Gln-Asp-Leu-
-Asn-Thr-Met-Leu-Asn-Thr-Val-Gly-Gly-His-Gln-Ala-Ala-
-Met-Gln-Met-Leu-Lys-Glu-Thr-Ile-Asn-Glu-Glu-Ala-Ala-
-Glu-Trp-Asp-Arg-Val-His-Pro-Val-His-Ala-Gly-Pro-Ile-
-Ala-Pro-Gly-Gln-Met-Arg-Glu-Pro-Arg-Gly-X-Z

d) H-X-Glu-Thr-Ile-Asn-Glu-Glu-Ala-Ala-Glu-Trp-Asp-Arg--Val-His-Pro-Val-X-Z

e) H-X-Asp-Arg-Val-His-Pro-Val-His-Ala-Gly-Pro-Ile-Ala--Pro-Gly-Gln-Met-Arg-Glu-Pro-Arg-Gly-Ser-Asp-Ile-Ala--Gly-Thr-Thr-Ser-Thr-Leu-Gln-Glu-Gln-Ile-Gly-Trp-Met--Thr-Asn-Asn-Pro-Pro-Ile-Pro-Val-Gly-Glu-Ile-Tyr-Lys--Arg-Trp-Ile-Ile-Leu-Gly-Leu-Asn-Lys-Ile-X-Z

f) H-X-Glu-Ile-Tyr-Lys-Arg-Trp-Ile-Ile-Leu-Gly-Leu-Asn--Lys-Ile-Val-Arg-Met-X-Z

g) H-X-Tyr-Lys-Arg-Trp-Ile-Ile-Leu-Gly-Leu-Asn-Lys-Ile--Val-Arg-Met-Tyr-Ser-Pro-Thr-Ser-Ile-Leu-Asp-Ile-Arg--Gln-Gly-Pro-Lys-Glu-Pro-Phe-Arg-Asp-Tyr-Val-Asp-Arg--Phe-Tyr-Lys-Thr-Leu-Arg-Ala-Glu-Gln-Ala-Ser-Gln-Glu--Val-Lys-Asn-Trp-Met-Thr-Glu-Thr-Leu-Leu-Val-Gln-Asn--Ala-Asn-Pro-Asp-Cys-Lys-Thr-Ile-Leu-Lys-Ala-Leu-Gly--X-Z

h) H-X-Thr-Glu-Thr-Leu-Leu-Val-Gln-Asn-Ala-Asn-Pro-Asp--Cys-Lys-Thr-Ile-Leu-Lys-Ala-Leu-Gly-Pro-Ala-Ala-Thr--Leu-Glu-Glu-Met-Met-Thr-Ala-Cys-Gln-Gly-Val-Gly-Gly--Pro-Gly-His-Lys-Ala-Arg-Val-Leu-Ala-Glu-Ala-Met-Ser--Gln-Val-Thr-Asn-Thr-Ala-Thr-Ile-Met-Met-X-Z, in both oxidized and unoxidized form, wherein in the oxidized form a bridge is formed between the two cysteines in the peptide

i) H-X-Lys-Ala-Leu-Gly-Pro-Ala-Ala-Thr-Leu-Glu-Glu-Met--Met-Thr-Ala-Cys-Gln-X-Z

in which X represents a chemical bond or a coupling-facilitating amino acid sequence, and Z represents $-NH_2$ or -OH.

The peptides according to the present invention are conveniently referred to as

a) HIV-1 hxb2 _gag_ 133-193
b) HIV-1 hxb2 _gag_ 143-156
c) HIV-1 hxb2 _gag_ 173-233
d) HIV-1 hxb2 _gag_ 203-218
e) HIV-1 hxb2 _gag_ 213-273
f) HIV-1 hxb2 _gag_ 260-276
g) HIV-1 hxb2 _gag_ 262-338
h) HIV-1 hxb2 _gag_ 318-378
i) HIV-1 hxb2 _gag_ 335-351

In another aspect the present invention relates to a diagnostic antigen having the ability of binding to antibodies which have a binding affinity for compounds containing an amino acid sequence corresponding to an epitope or clusters of epitopes of HIV-1 p24.

In yet another aspect the present invention relates to a method of discriminating between a false and true diagnosed HIV-1 positive serum sample from a subject, characterised in that at least one diagnostic antigen having the ability of binding to antibodies which have a binding affinity for compounds containing an amino acid sequence corresponding to an epitope or clusters of epitopes of HIV-1 p24, optionally coupled to a carrier, is added to the serum sample, and that possible antigen/antibody complexes formed are detected using an immunoassay, whereby the discrimination is established based upon that said serum sample is false HIV-1 positive if said complexes are detected, and true HIV-1 positive if said complexes are not detected.

In a preferred embodiment of the invention said diagnostic antigen is a diagnostic antigen according to the invention.

Other characteristics and features of the present invention appears from the attached claims.

Among the AIDS-inducing retro viruses so far known, the most common in the world is HIV-1, while the partially structurally different HIV-2 is mainly restricted to West Africa. The retro viruses HTLV-1 and HTLV-2 do not induce AIDS, but AIDS-related diseases, for example T-cell leukemia. The present invention is therefore concerned with HIV-1 and amino acid sequences unique for the HIV-1 proteins are contemplated.

The interior parts, for example the nucleocapsid or the core containing the RNA, of the HIV-1 viruses are protected by an envelope. The interior parts, called gag (group-specific antigen) and the envelope, called env, are built up of different proteins. These proteins, i.e. the precursors, are predominant in the beginning of the virus life cycle, while others are predominant in later phases.

The following list shows major genes and gene products of HIV-1.

Gene	Gene products/proteins
group-specific antigen/core (gag)	p17, p24, p55
polymerase (pol)	p31, p51, p66
envelope (env)	gp41, gp120, gp160

p = protein

gp = glycoprotein

The numbers indicate the approximate molecular weights of the proteins expressed in kilodaltons.

p55 is a precursor of p24 and p17.

gp160 is a precursor of gp120 and gp41.

At a certain stage of the virus life cycle, an enzyme cleaves the p55 molecule from the p24 molecule. Thus, the whole p24 protein constitutes a part of the original p55 protein. As the present invention relates to peptides with the ability to react with antibodies induced by an amino

acid sequence corresponding to an epitope or clusters of epitopes of HIV-1 p24, p55 is also included in this definition. Thus, throughout the present specification and claims, the expression "an epitope or clusters of epitopes of HIV-1 p24" used, is intended to include the same epitope or epitopes of HIV-1 p55 as well.

The peptides according to the present invention have ability to react with antibodies raised against an amino acid sequence corresponding to or closely related to an epitope or clusters of epitopes of the HIV-1 p24 protein.

All these new peptides and/or parts thereof react in a uniquely specific way with the above mentioned non HIV-1 antibodies.

In the peptides according to the present invention, X represents a chemical bond or a sequence of at least 4, and preferably 8, particular amino acid residues, which each are chosen from the group consisting of -Thr- and -Ser-. When X is an amino acid sequence, it can be located either in the C- or N-terminus of the peptides but not in both ends at the same time. If it is for example located in the C-terminus, X in the N-terminus corresponds to a chemical bond and vice versa. However, X can be a bond at both ends of the peptide according to the present invention. Said amino acid sequence acts as a coupling facilitating spacer, which permits proper bindning to the carrier to which the peptides according to the present invention will be bound during the discrimination method. The sequence X should not be an amino acid sequence which adversely affects the result of the diagnosis method. Accordingly, it should not have a too high charge or be too hydrophobic and it should not disturb the conformation of the peptides. The amino acids threonine and serine also fulfill these requirements particularily well, and any one of the amino acids in said sequence X can be threonine or serine. The number of amino acids in this spacer sequence should be at least 4, but in a preferred embodiment according to the present invention 8 amino acids are used.

The polypeptides according to the present invention can be bound via the amino acid sequence X to a carrier by physical/chemical interaction, as for example covalent binding, ionic binding, hydrogen binding or hydrophobic binding. Examples of covalent binding are esther, ether and disulfide binding.

The expression "carrier" used herein should be interpreted broadly, and it can be any material which is compatible with and not negatively affects the method according to the present invention, for example resins, microplates, plastic surfaces, gels, matrixes etc.

The expression "epitope" used herein means antigenic or immunogenic determinant and relates to a specific part of a structure of an antigen inducing an immuno response, and the produced antibodies are directed against this part.

The immuno assay method according to the present invention can be enzyme immunoassay (EIA), radioimmuno assay (RIA), immunoassay involving metal labelling, fluorescence immunoassay (FIA) or an immunoassay in which the peptide is soluble and inhibits another reaction.

As stated above, the peptides according to the present invention have ability to bind to antibodies raised against amino acid sequences corresponding to an epitope or clusters of epitopes of HIV-1 p24. Thus, these antibodies are not raised against HIV-1 proteins. It is reasonable to assume that p24 antibody reactions are due either to coincidental or selection controlled antigenic mimicry between the C-terminus part of the p24 amino acid sequence and an unknown non-HIV-epitope. The origin may be autoimmunity or immunization with a retrovirus other than HIV.

Brief description of the Drawings

Figure 1. Distribution of absorbance differences in EIA with synthetic peptides derived from HIV-1 *gag*.

Figure 2. Patterns of reactivity with six long synthetic peptides derived from HIV-1 p24.

Figure 3. Electrophoretic immunoblotting with HIV-1 antigen of one p24 EIB and HIV-1 gag 318-378 reactive human serum (number I, Table 3) before and after threefold absorption "in-well" with solid-phase-bound HIV-1 gag 318-378 (A), HTLV-I gag 118-140 (B), or wells mock-coated with PBS (C).

EXPERIMENTS

Identification of HIV-1 p24 reactive sera

First, a standard whole virus HIV-1 antibody screening test, the Organon Vironostica-anti-HIV (Organon Technica), is performed. Serum samples are allowed to react with whole HIV-1 virus bound to a solid phase. Possible binding of antibodies to the different parts of the virus is detected by measuring the absorbance of the sample. Samples showing an absorbance above a predetermined cut off value are deemed to be positive.

In the present case about 150 000 healthy persons in the region of Southern Sweden were tested, and 785 were regarded positive. Blood donors, pregnant women and heterosexuals worrying about HIV, but essentially without risk behaviour, provided the great majority of these samples.

Subsequently these 785 positive sera were subjected to a confirmatory electroimmuno blotting (EIB) assay, i.e. a Western blot (described above). Nine of these 785 samples showed a single p24 pattern of serological activity. Among the persons donating the 9 sera were 5 women, 3 men, and 1 of unknown sex. The ages were 22, 24, 24, 24, 25, 33, 36 and 57. The age was unknown for one person. The reasons for making the HIV antibody test stated on the request form were: blood donor 4, pregnancy 3 and unknown 2. The persons were not contacted themselves, because it was not necessary to trouble them at this stage of the investigation concerning the nature of the false-positive HIV serological reactions.

Twenty confirmed HIV-1 positive sera and 17 sero-negative unselected Swedish blood donors were used as controls. When reactivities were found with three or two (HIV-1 gag 318-378 oxidized and unoxidized and HIV-1 gag 262-338) of the peptides, their origin were reinvestigated. They proved to be early sera from patients who shortly afterwards seroconverted to full EIB positivity. These sera were consequently not included in the study.

Internationally accepted criteria for judgement of HIV-1 seropositivity (See The Consortium for Retrovirus Serology Standardization. Serological diagnosis of human immunodeficiency virus infection by Western Blot testing. JAMA 260:674-679 (1988) and Centers for Disease Control: Interpretation and use of the Western blot assay for serodiagnosis of human immunodeficiency virus type 1 infections. MMWR 1989, 38:1-7) were followed.

Immunoassays

Four commercial assays were used: A whole virus HIV-1 antibody test, Organon Vironostica anti-HIV (Organon Technica), the Abbott HIV-1 antibody EIA where recombinant antigen has been prepared from bacteria, the Abbott ENVACORE confirmatory HIV-1 antibody competition antibody EIA and the Du Pont Biotech HIV-1 electrophoretic immunoblot (EIB) system. They were used according to the instructions of the manufacturers.

EIB strips were measured with a Bio-Rad 620 Video Densitometer (Richmond, California, USA) (see Blomberg J, Klasse PJ: Quantification of immunoglobulin G on electrophoretic immunoblot strips as a tool for human immunodeficiency virus serodiagnosis. J Clin Microbiol 1988, 26:111-115).

Peptide EIA:s were performed approximately as described (see Blomberg J, Nilsson I, Andersson M: Viral antibody screening system that uses a standardized single dilution immunoglobulin G enzyme immunoassay with multiple antigens. J Clin Microbiol 1983, 17:1081-1091 and Klasse PJ, Pipkorn R, Blomberg J: Presence of antibodies to a

putatively immunosuppressive part of human immunodeficiency virus (HIV) envelope glycoprotein gp41 is strongly associated with health among HIV-positive subjects. Proc Natl Acad Sci USA 1988, 85:5225-5229). Briefly, 100 µl of 20 µl/ml of peptide in PBS-M (PBS: 8.0 g NaCl, 0.2 g KH_2PO_4, 1.4 g $Na_2HPO_4 \cdot 2H_2O$, 0.2 g KCl per liter) (PBS-M: PBS with 10 µl/ml of sodium merthiolate) was allowed to bind first for 2-6 h at room temperature, then overnight at 4°C. 200 µl of blocking solution (4% bovine serum albumin (BSA)/0.2% gelatin/0.05% Tween 20) were added, allowed to bind for 2-6 h at room temperature, then over night at 4°C, and frozen. At the day of use, the plates were thawed and washed three times in washing fluid (freshly made 0.05% Tween 20 in PBS). 100 µl of appropriately diluted control sera and test sera diluted 1/50 in diluent I (3% BSA, 0.2% gelatin, 0.05% Tween 20 in PBS-M) were then added and the plates were incubated with shaking at room temperature for 1 h. After another wash, 100 µl biotinylated affinity purified goat anti-human IgG (Sigma) diluted 1/1500 in diluent II (0.2% gelatin in PBS-M) were added, and incubated with shaking for 1 h at room temperature. After washing, 100 µl avidin-peroxidase (Sigma) diluted 1/300 in diluent II were added, and the plates were incubated with shaking for 1 h at room temperature. The plates were washed thoroughly and beaten dry. 100 µl substrate solution (20 mg o-phenylenediamine + 10 µl 30% H_2O_2 in color buffer, made fresh each day) (Color buffer: 34.7 mM citric acid, 66.7 mM Na_2HPO_4, pH 5.0), was added, and the plates were incubated in the dark at room temperature for 30 min and read at 450 nm in a microplate photometer.

Synthetic peptides

The following peptides were used in the experiments:

Table 1. Synthetic peptides employed in the present study. The arrows point to the cysteine residues which we tried to join by oxidation.

```
HIV-1 hxb2 gag 133-193 PIVQNIQGQMVHQAISPRTLNAWVKVVEEKAFSPEVIPMFSALSEGATPQDLNTMLNTVGG
HIV-1 hxb2 gag 143-156           VHQAISPRTLNAWV

HIV-1 hxb2 gag 173-233 SALSEGATPQDLNTMLNTVGGHQAAMQMLKETINEEAAEWDRVHPVHAGPIAPQMREPRG
HIV-1 hxb2 gag 203-218                               ETINEEAAEWDRVHPV
HIV-1 hxb2 gag 210-230                                      AEWDRVHPVHAGPIAPGQMRE

HIV-1 hxb2 gag 213-273 DRVHPVHAGPIAPGQMREPRGSDIAGTTSTLQEQIGWMTNNPPIPVGEIYKRWIILGLNKI
HIV-1 hxb2 gag 223-243           IAPGQMREPRGSDIAGTTSTL
HIV-1 hxb2 gag 260-276                                                EIYKRWIILGLNKIVRM

HIV-1 hxb2 gag 262-338 YKRWIILGLNKIVRMYSPTSILDIRQGPKEPFRDYVDRFYKTLRAEQASQEVKNWMTETLLVQNANPDCKTILKALG
HIV-1 hxb2 gag 284-300                       DIRQGPKEPFRDYVDRF

                                   330                 350
                                   V                   V
HIV-1 hxb2 gag 318-378 TETLLVQNANPDCKTILKALGPAATLEEMMTACQGVGGPGHKARVLAEAMSQVTNTATIMM
HIV-1 hxb2 gag 335-351                  KALGPAATLEEMMTACQ
HIV-1 hxb2 gag 348-373                               TACQGVGGPGHKARVLAEAMSQVTN
HIV-1 hxb2 gag 359-382                                          KARVLAEAMSQVTNSATIMMQRGN
```

As appears from the list above, some shorter fragments of the longer peptides according to the present invention were used in the experiments. Of these shorter peptides, only HIV-1 hxb2 gag 143-156, 203-218, 260-276 and 335-351 are comprised in the present invention. The amino acid sequences of the peptides are expressed herein with the one letter symbol system in an attempt to in a more simple way show the relation between the longer peptides and the shorter fragments thereof.

Both the short and long peptides (17-77 aa) were used in an attempt to catch as much antibody reactivity as possible.

The peptides were synthesized using Fmoc chemistry on an Applied Biosystems 430A solid phase automatic peptide synthesizer. They were subsequently purified by reverse-phase HPLC on a C18 column. Their purity was 95% according to an analytical HPLC procedure. The peptide HIV-1 gag 318-378 was tested both in oxidized and unoxidized form.

According to the invention, the peptides can be used as a diagnostic antigen either solved in the serum sample or bound to a carrier. Hereinafter, some modes of using the diagnostic antigen according to the present invention will be described.

EXPERIMENTS

Reactivity of p24 reactive sera in commercial HIV-1 antibody tests

In this experiment three commercial antibody EIA:s were used, i.e. the above specified Organon Vironostica anti-HIV, Abbott HIV-1 antibody EIA and Abbott ENVACORE confirmatory HIV-1 antibody competition antibody EIA. As stated above, the nine HIV-1 p24 reactive sera were derived from 785 repeatedly HIV-1 antibody screening test positive sera. These were in their turn derived from around 150 000 sera subjected to HIV-1 antibody screening tests at primary test sites. The distribution of reactivities in the nine sera with p24 bands in HIV-1 EIB can be seen in Table 2.

TABLE 2

Characteristics of the nine HIV-1 p24 reactive sera analysed, and the patients donating them

Serum no	Reactive in EIA with long peptide	Organon whole virus HIV-1 EIA (A 492)	Abbott recombinant HIV-1 EIA (A 492)	EIB pattern	Sex	Age	Reason for testing
A	262-338	0.594/0.194	0.061/0.122	p24	F	25	Pregnancy
B	133-193	0.123/0.162	0.064/0.308	p24	F	24	Blood donor
C	133-193	0.141/0.130	0.178/0.357	p24	M	22	Blood donor
D	133-193	0.279/0.130	0.040/0.234	p24	F	24	Blood donor
E	133-193	0.209/0.137	0.109/0.244	p24+p55	M	?	Unknown
F	318-378	0.056/0.109	0.448/0.216	p24+p55	F	33	Pregnancy
G	none	0.052/0.123	0.158/0.396	p24	M	24	Blood donor
H	318-378	0.090/0.154	0.282/0.251	p24	F	36	Pregnancy
I	none	0.165/0.129	0.048/0.320	p24	F	57	Unknown

As appears from Table 2, five sera reacted only in the Organon whole virus EIA, while two reacted only in the Abbott recombinant HIV-1 antigen EIA. If the value on the left of the slanting line in the Table is higher than the value on the right side, the serum is considered to have been reacting. The remaining two had been reactive in the Organon test at the remitting test site, but were not positive in the laboratory of the present inventor. None reacted in the Abbott ENVACORE competition EIA (not shown). Sera which were reactive in the Organon test came from test sites which used the Organon tests, and those who reacted in the Abbott recombinant test came from the sites which employed the same test. As further appears from the Table, three of five Organon-reactive sera were positive in EIA with peptides gag 133-193 and one positive with 262-338 according to the present invention. These four sera were negative in Abbott HIV-1 EIA, wheras the two Abbott recombinant reactive sera were positive in EIA

with peptide gag 318-378 according to the invention and negative in the Organon whole virus EIA. Thus, the pattern of reactivity in the commercial anti-HIV tests may be an indication of what portion of the p24 molecule the anti-p24 antibodies are directed to.

Reactivity of p24 positive sera with syntetic peptides derived from p24. Comparison with confirmed HIV-1 antibody positive and negative blood donor sera

The distribution of IgG reactivities, i.e. the absorbance differences, in EIA of HIV-1 p24 antibody positive sera on five long and ten shorter p24 derived syntetic peptides is shown in Figure 1a, 1b and 1c. The sera in these runs were a) the nine p24 reactive sera, b) 20 confirmed HIV-1 antibody positive sera and c) 17 HIV sero-negative blood donors. In Table 3 the frequence of reactivity (absorbance difference >0.4) is shown with synthetic peptides derived from the HIV p24 sequence.

Table 3. Frequency of rectivity (absorbance difference >0.4) with synthetic peptides derived from the HIV p24 sequence.

Peptide	Frequency with Swedish p24+/-p55 reactive sera	Frequency with Swedish confirmed HIV-1 positive sera	Frequency with Swedish sero-negative blood donor sera
	N=9	N=20	N=17
HIV-1 hxb2 gag 133-193	5	3	0
HIV-1 hxb2 gag 143-156	0	2	0
HIV-1 hxb2 gag 173-233	0	1	0
HIV-1 hxb2 gag 203-218	0	1	0
HIV-1 hxb2 gag 210-230	0	0	0
HIV-1 hxb2 gag 213-273	0	6	1
HIV-1 hxb2 gag 223-243	0	0	0
HIV-1 hxb2 gag 260-276	0	3	0
HIV-1 hxb2 gag 262-338	1	3	3
HIV-1 hxb2 gag 284-300	0	0	0
HIV-1 hxb2 gag 318-378	2	15	0
same, in oxidized form	1	15	0
HIV-1 hxb2 gag 335-351	0	5	0
HIV-1 hxb2 gag 348-373	0	0	0
HIV-1 hxb2 gag 359-382	0	0	0

According to Fig 1a four p24 positive sera reacted, two strongly, with the N-terminal long peptide HIV-1 gag 133-193, one serum with HIV-1 gag 262-338 and two with the C-terminal HIV-1 gag 318-378 in oxidized form. One serum had a reactivity with gag 133-193 just below the stipulated cut-off absorbance difference of 0.4. None of the p24 positive sera reacted with one of the shorter HIV-1 gag peptides.

In contrast, 15 of 20 confirmed HIV-1 positive sera reacted most strongly with peptide 318-378 both in unoxidized and oxidized form (Fig 1b) and to lesser extents with the other long p24 derived peptides. The sera from HIV-1 seronegative Swedish blood donors were largely unreactive with all the peptides (Fig 1c). However, peptides 213-273 and 262-338 reacted significantly also with a few blood donor sera, and results obtained with them thus had to be interpreted with caution.

Neither of the false HIV antibody positive nor of the blood donor sera reacted with the ten shorter p24 derived peptides. Five of the confirmed HIV antibody positive sera reacted with HIV-1 gag 335-351, three with HIV-1 gag 260-276, two with HIV-1 gag 143-156 and one with HIV-1 gag 203-218 (see Table 3). Thus the shorter peptides were not useful for demonstration of false positive HIV antibodies, but were useful for further delineation of some of the p24 epitopes recognized by HIV-1 infected persons.

The frequencies of reactivity with long synthetic p24 derived peptides are shown in Table 4.

TABLE 4

Frequency of reactivity with long synthetic peptides derived from HIV-1 p24

Peptide	Group			Probability (Fisher exact test)		
HIV gag	p24+/-p55 positive (FP)	Confirmed positive (TP)	Seroneg blood donors (BD)	FP/TP	FP/BD	TP/BD
133-193	4/9	3/20	0/17	0.10	0.008	ns
173-233	0/9	1/20	0/17	ns	ns	ns
213-273	0/9	5/20	1/17	0.13	ns	0.13
262-338	1/9	3/20	3/17	ns	ns	ns
318-378	2/9	15/20	0/17	0.01	0.11	0.000001
318-378 ox	0/9	15/20	0/17	0.0002	ns	0.000001

Besides the expected significantly higher reactivity of confirmed HIV antibody positive sera versus blood donor or false positive sera, a significant difference between false positive sera and blood donors was evident with peptide HIV-1 gag 133-193 (p=0.008). The difference between false positive and blood donor sera for peptides HIV-1 gag 318-378 (unoxidized) and HIV-1 gag 262-338 only reached a borderline significance (p=0.11) or was not significant, respectively. The pattern of reactivity with the six long p24 peptides is shown in Fig 2, in which the hatched areas show an absorbance difference larger than 0.4. The number of sera wich displayed a certain reactivity pattern is shown under "No". FP = p24 +/- p55 reactive (false-positive) sera. TP = confirmed HIV-1 antibody positive sera. BD = HIV-1 seronegative blood donor sera. The peptide number codes are evident from Table 1.

Absorption of p24 positive sera with immobilized peptides

p24 antibody positive sera were diluted to yield an absorbance increment versus the negative control well of 0.5-1.0 in EIA dilution fluid (3% BSA, 0.2% gelatin in PBS-M). Sera were incubated in wells precoated with p24 peptides for 1 h. The supernatant was then transferred to a neighbouring well, and incubated for another hour. This was repeated another time. A total of three one hour rounds of absorptions were thus made. The supernatant was then diluted 1/2 in blotting solution and transferred to an EIB well and allowed to react in an HIV-1 EIB (DuPont) under otherwise standard conditions. In most experiments wells coated with the EIA reactive p24 peptide together with the following controls were used: Wells coated with an unreactive p24 peptide, a non-HIV peptide (HTLV-I gag 118-140) and wells mock-coated with PBS in parallel. The intensity of the p24 band was measured by reflectance densitometry on the EIB strips (see Blomberg J, Klasse PJ: Quantification of immunoglobulin G on electrophoretic immunoblot strips as a tool for human immunodeficiency virus serodiagnosis. J Clin Microbiol 1988, 26:111-115 and Blomberg J, Klasse PJ: Specificities and sensitivities of three systems for determination of antibodies to human immunodeficiency virus by electrophoretic immunoblotting. J Clin Microbiol 26:106-110 (1988)).

To ascertain that the peptide EIA reactivities really were directed against p24 of HIV-1, six of the eight peptide-reactive "p24 +/- p55" reactive sera (nr A-E and H in Table 2) were incubated with p24 derived and control peptides in wells of EIA microplates in three successive one hour absorptions. We ascertained that an absorption of peptide-reactive antibody had taken place by running an EIA in the same wells after the completion of the absorptions. The remaining antibodies were then studied in a standard overnight HIV-1 EIB. In two (nr A and H) of the six sera the anti-p24 activity was visibly diminished by absorption with the immobilized peptide which had reacted

strongly in EIA. One of these two sera were reactive with and absorbed by peptide 262-338 and one with peptide 318-378. The anti-p24 EIB bands were not diminished after absorption with another, HIV or HTLV derived, immobilized peptide or with mock-coated wells. (cf Fig 3, in which "+" = confirmed HIV antibody positive control. "-" = HIV antibody negative serum control. The arrows indicate positions of HIV-1 proteins.) Thus, the argument for a localization of the antibody reactivity to these two portions of p24 was strengthened in two of six peptide-reactive p24 +/- p55 positive sera subjected to peptide absorption.

The long peptides were chosen to optimize serological reactivity with HIV gag antibodies. Long peptides simulate conformational (non-continuous) epitopes of HIV-1 p24 gag particularly well. Such long peptides have to our knowledge never before been used for HIV serology. Thus, the long peptides, especially the HIV-1 gag 133-193 and 318-378 were excellent reagents for both discrimination of true from false HIV-1 antibody reactivity and for detection of both types of reactivity. In order to obtain a maximum discriminatory effect between false and positive reactivities, preferably a combination of several peptides according to the present invention are used as diagnostic antigens.

CLAIMS

1. Peptides of the formula

a) H-X-Pro-Ile-Val-Gln-Asn-Ile-Gln-Gly-Gln-Met-Val-His--Gln-Ala-Ile-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys--Val-Val-Glu-Glu-Lys-Ala-Phe-Ser-Pro-Glu-Val-Ile-Pro--Met-Phe-Ser-Ala-Leu-Ser-Glu-Gly-Ala-Thr-Pro-Gln-Asp--Leu-Asn-Thr-Met-Leu-Asn-Thr-Val-Gly-Gly-X-Z

b) H-X-Val-His-Gln-Ala-Ile-Ser-Pro-Arg-Thr-Leu-Asn-Ala--Trp-Val-X-Z

c) H-X-Ser-Ala-Leu-Ser-Glu-Gly-Ala-Thr-Pro-Gln-Asp-Leu--Asn-Thr-Met-Leu-Asn-Thr-Val-Gly-Gly-His-Gln-Ala-Ala--Met-Gln-Met-Leu-Lys-Glu-Thr-Ile-Asn-Glu-Glu-Ala-Ala--Glu-Trp-Asp-Arg-Val-His-Pro-Val-His-Ala-Gly-Pro-Ile--Ala-Pro-Gly-Gln-Met-Arg-Glu-Pro-Arg-Gly-X-Z

d) H-X-Glu-Thr-Ile-Asn-Glu-Glu-Ala-Ala-Glu-Trp-Asp-Arg--Val-His-Pro-Val-X-Z

e) H-X-Asp-Arg-Val-His-Pro-Val-His-Ala-Gly-Pro-Ile-Ala--Pro-Gly-Gln-Met-Arg-Glu-Pro-Arg-Gly-Ser-Asp-Ile-Ala--Gly-Thr-Thr-Ser-Thr-Leu-Gln-Glu-Gln-Ile-Gly-Trp-Met--Thr-Asn-Asn-Pro-Pro-Ile-Pro-Val-Gly-Glu-Ile-Tyr-Lys--Arg-Trp-Ile-Ile-Leu-Gly-Leu-Asn-Lys-Ile-X-Z

f) H-X-Glu-Ile-Tyr-Lys-Arg-Trp-Ile-Ile-Leu-Gly-Leu-Asn--Lys-Ile-Val-Arg-Met-X-Z

g) H-X-Tyr-Lys-Arg-Trp-Ile-Ile-Leu-Gly-Leu-Asn-Lys-Ile--Val-Arg-Met-Tyr-Ser-Pro-Thr-Ser-Ile-Leu-Asp-Ile-Arg--Gln-Gly-Pro-Lys-Glu-Pro-Phe-Arg-Asp-Tyr-Val-Asp-Arg--Phe-Tyr-Lys-Thr-Leu-Arg-Ala-Glu-Gln-Ala-Ser-Gln-Glu--Val-Lys-Asn-Trp-Met-Thr-Glu-Thr-Leu-Leu-Val-Gln-Asn-

-Ala-Asn-Pro-Asp-Cys-Lys-Thr-Ile-Leu-Lys-Ala-Leu-Gly- -X-Z

h) H-X-Thr-Glu-Thr-Leu-Leu-Val-Gln-Asn-Ala-Asn-Pro-Asp- -Cys-Lys-Thr-Ile-Leu-Lys-Ala-Leu-Gly-Pro-Ala-Ala-Thr- -Leu-Glu-Glu-Met-Met-Thr-Ala-Cys-Gln-Gly-Val-Gly-Gly- -Pro-Gly-His-Lys-Ala-Arg-Val-Leu-Ala-Glu-Ala-Met-Ser- -Gln-Val-Thr-Asn-Thr-Ala-Thr-Ile-Met-Met-X-Z, in both oxidized and unoxidized form, wherein in the oxidized form a bridge is formed between the two cysteines in the peptide

i) H-X-Lys-Ala-Leu-Gly-Pro-Ala-Ala-Thr-Leu-Glu-Glu-Met- -Met-Thr-Ala-Cys-Gln-X-Z

in which X represents a chemical bond or a coupling-facilitating amino acid sequence, and Z represents $-NH_2$ or -OH.

2. Peptide according to claim 1, wherein the coupling-facilitating amino acid sequence represents at least 4, preferably 8, amino acid residues, which each is chosen from the group consisting of -Thr- and -Ser-.

3. A diagnostic antigen having the ability of binding to antibodies which have a binding affinity for compounds containing an amino acid sequence corresponding to an epitope or clusters of epitopes of HIV-1 p24, characterised in that it mainly consists of an antigen, which antigen is selected from peptides comprising the amino acid sequence

a) H-X-Pro-Ile-Val-Gln-Asn-Ile-Gln-Gly-Gln-Met-Val-His- -Gln-Ala-Ile-Ser-Pro-Arg-Thr-Leu-Asn-Ala-Trp-Val-Lys- -Val-Val-Glu-Glu-Lys-Ala-Phe-Ser-Pro-Glu-Val-Ile-Pro- -Met-Phe-Ser-Ala-Leu-Ser-Glu-Gly-Ala-Thr-Pro-Gln-Asp- -Leu-Asn-Thr-Met-Leu-Asn-Thr-Val-Gly-Gly-X-Z

b) H-X-Val-His-Gln-Ala-Ile-Ser-Pro-Arg-Thr-Leu-Asn-Ala-

-Trp-Val-X-Z

c) H-X-Ser-Ala-Leu-Ser-Glu-Gly-Ala-Thr-Pro-Gln-Asp-Leu--Asn-Thr-Met-Leu-Asn-Thr-Val-Gly-Gly-His-Gln-Ala-Ala--Met-Gln-Met-Leu-Lys-Glu-Thr-Ile-Asn-Glu-Glu-Ala-Ala--Glu-Trp-Asp-Arg-Val-His-Pro-Val-His-Ala-Gly-Pro-Ile--Ala-Pro-Gly-Gln-Met-Arg-Glu-Pro-Arg-Gly-X-Z

d) H-X-Glu-Thr-Ile-Asn-Glu-Glu-Ala-Ala-Glu-Trp-Asp-Arg--Val-His-Pro-Val-X-Z

e) H-X-Asp-Arg-Val-His-Pro-Val-His-Ala-Gly-Pro-Ile-Ala--Pro-Gly-Gln-Met-Arg-Glu-Pro-Arg-Gly-Ser-Asp-Ile-Ala--Gly-Thr-Thr-Ser-Thr-Leu-Gln-Glu-Gln-Ile-Gly-Trp-Met--Thr-Asn-Asn-Pro-Pro-Ile-Pro-Val-Gly-Glu-Ile-Tyr-Lys--Arg-Trp-Ile-Ile-Leu-Gly-Leu-Asn-Lys-Ile-X-Z

f) H-X-Glu-Ile-Tyr-Lys-Arg-Trp-Ile-Ile-Leu-Gly-Leu-Asn--Lys-Ile-Val-Arg-Met-X-Z

g) H-X-Tyr-Lys-Arg-Trp-Ile-Ile-Leu-Gly-Leu-Asn-Lys-Ile--Val-Arg-Met-Tyr-Ser-Pro-Thr-Ser-Ile-Leu-Asp-Ile-Arg--Gln-Gly-Pro-Lys-Glu-Pro-Phe-Arg-Asp-Tyr-Val-Asp-Arg--Phe-Tyr-Lys-Thr-Leu-Arg-Ala-Glu-Gln-Ala-Ser-Gln-Glu--Val-Lys-Asn-Trp-Met-Thr-Glu-Thr-Leu-Leu-Val-Gln-Asn--Ala-Asn-Pro-Asp-Cys-Lys-Thr-Ile-Leu-Lys-Ala-Leu-Gly--X-Z

h) H-X-Thr-Glu-Thr-Leu-Leu-Val-Gln-Asn-Ala-Asn-Pro-Asp--Cys-Lys-Thr-Ile-Leu-Lys-Ala-Leu-Gly-Pro-Ala-Ala-Thr--Leu-Glu-Glu-Met-Met-Thr-Ala-Cys-Gln-Gly-Val-Gly-Gly--Pro-Gly-His-Lys-Ala-Arg-Val-Leu-Ala-Glu-Ala-Met-Ser--Gln-Val-Thr-Asn-Thr-Ala-Thr-Ile-Met-Met-X-Z

i) H-X-Lys-Ala-Leu-Gly-Pro-Ala-Ala-Thr-Leu-Glu-Glu-Met--Met-Thr-Ala-Cys-Gln-X-Z

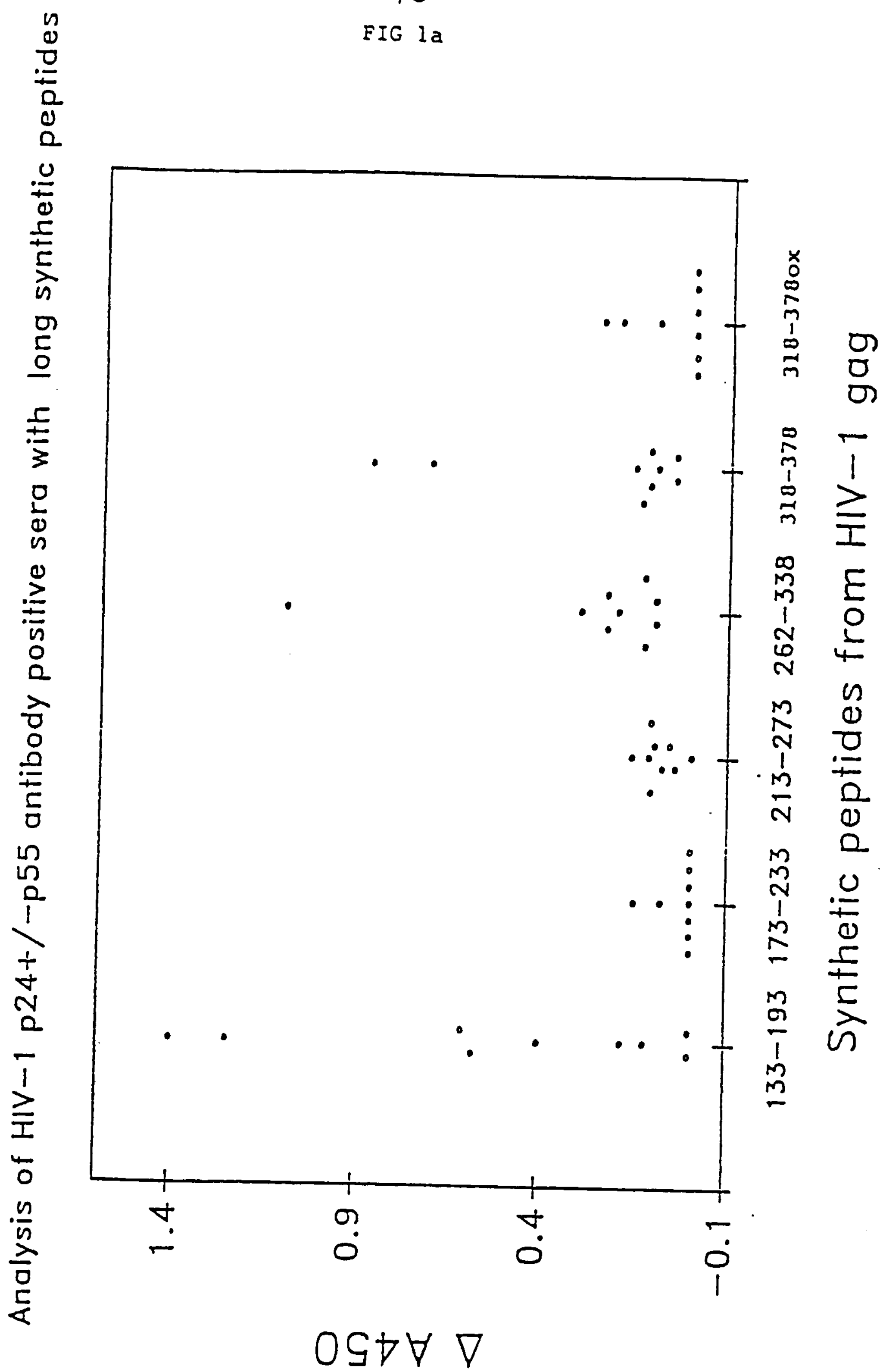
or antigenic parts thereof, wherein one X represents a chemical bond and the other X represents a chemical bond or a coupling-facilitating amino acid sequence and Z represents $-NH_2$ or -OH.

4. A method of discriminating between a false and true diagnosed HIV-1 positive serum sample, which first has been diagnosed as positive in a standard HIV-1 antibody screening EIA test and then shown to exhibit a p24 pattern of serological activity in an electrophoretic immunoblot assay, characterised in that at least one diagnostic antigen having the ability of binding to antibodies which have a binding affinity for compounds containing an amino acid sequence corresponding to an epitope or clusters of epitopes of HIV-1 p24, optionally coupled to a carrier, is added to said serum sample, and that possible antigen/antibody complexes formed are detected using an immunoassay, whereby the discrimination is established based upon that said serum sample is false HIV-1 positive if said complexes are detected, and true HIV-1 positive if said complexes are not detected.

5. A method according to claim 4, characterised in that the immunoassay for detection of said antibody/antigen complex is an enzyme immunoassay (EIA), radioimmuno assay (RIA), immunoassay involving metal labelling, fluorescence immunoassay (FIA) or an immunoassay in which the peptide is soluble and inhibits another reaction.

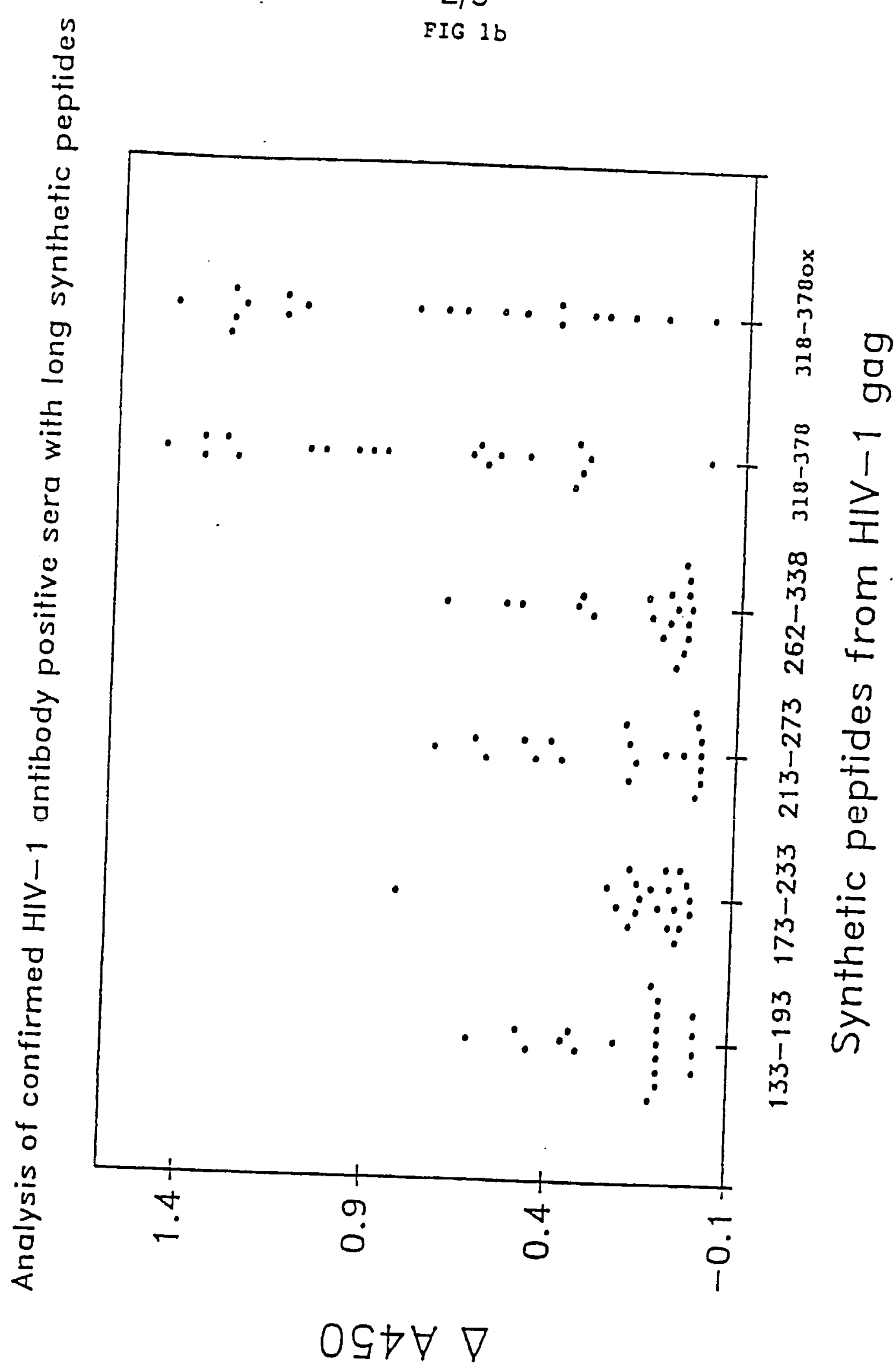
6. A method according to claim 4, characterised in that the carrier is a resin, microplate, plastic surface, gel or matrix.

FIG 1a


Analysis of HIV-1 p24+/-p55 antibody positive sera with long synthetic peptides
Δ A450
1.4
0.9
0.4
-0.1
133-193
173-233
213-273
262-338
318-378
318-378ox
Synthetic peptides from HIV-1 gag

2/5

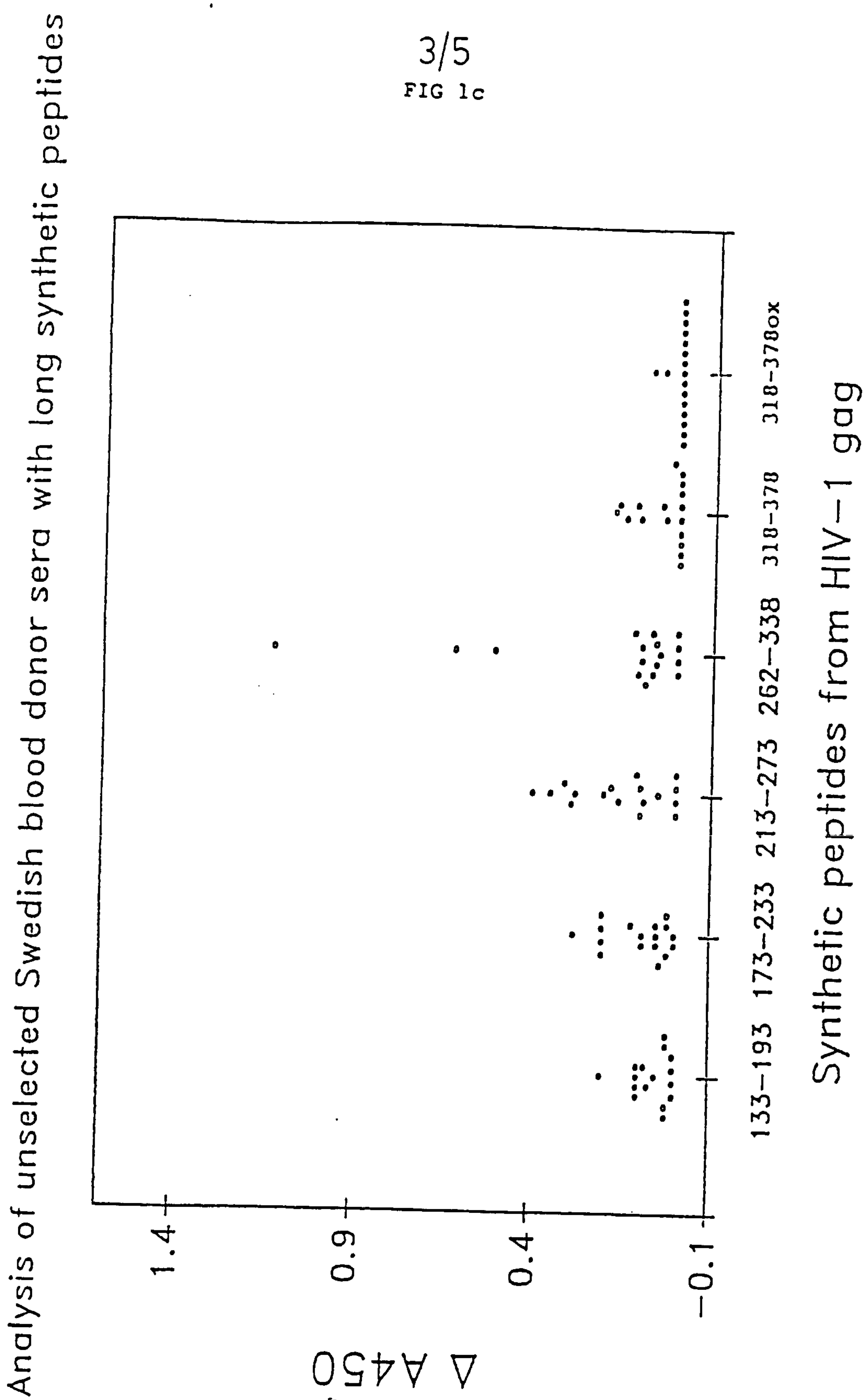
FIG 1b



SUBSTITUTE

3/5

FIG 1c



SUBSTITUTE

FIG 2

PATTERN OF REACTIVITY WITH LONG p24 PEPTIDES

SERA

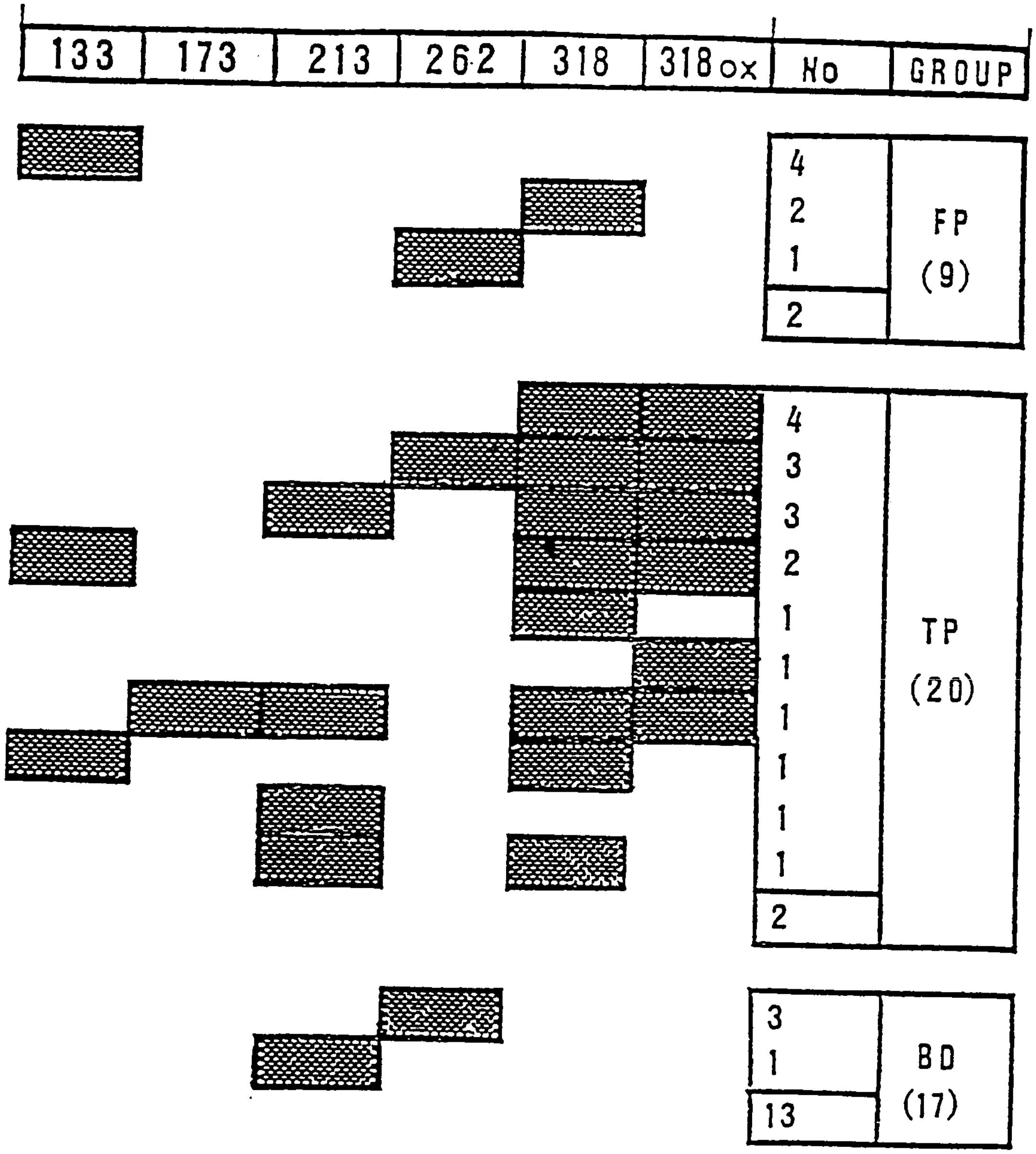
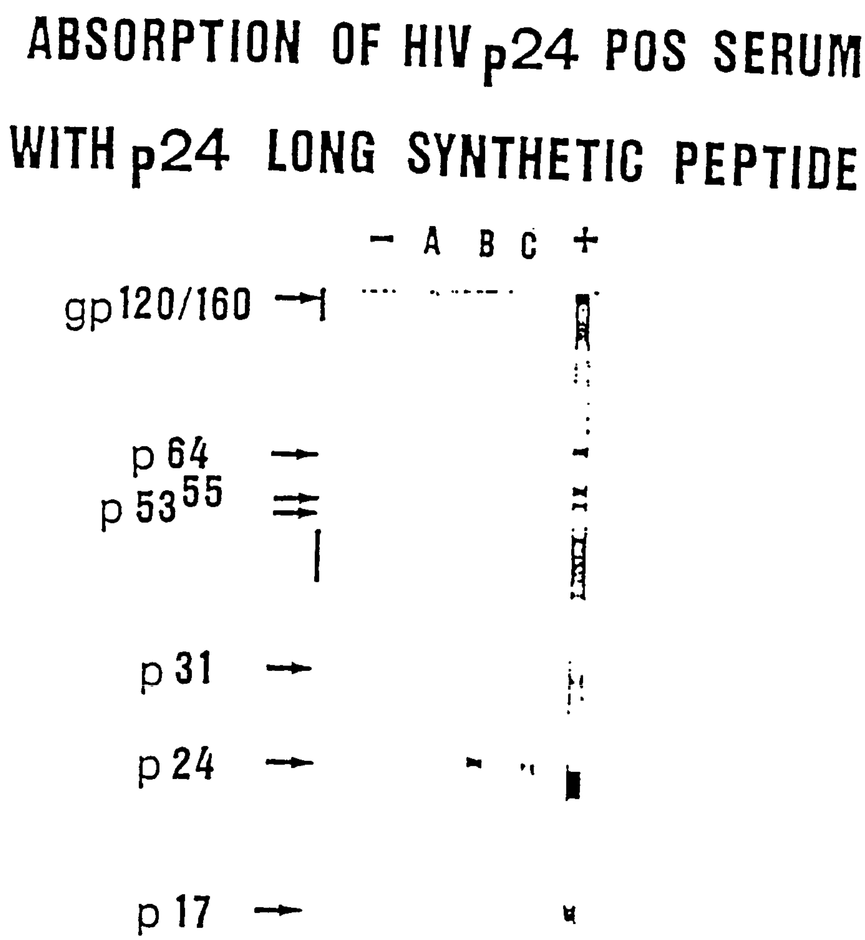


FIG 3


ABSORPTION OF HIV p24 POS SERUM
WITH p24 LONG SYNTHETIC PEPTIDE
− A B C +
gp120/160
p 64
p 53 55
p 31
p 24
p 17

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE 91/00114

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: G 01 N 33/569, C 07 K 7/04

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	G 01 N; C 07 K; A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
Y	EP, A2, 0330359 (BIO-RAD LABORATORIES, INC.) 30 August 1989, see page 10, lines 5-15 and claims	1,3-6
X		1F,3F
	--	
Y	WO, A1, 8606414 (GENETIC SYSTEMS CORPORATION) 6 November 1986, see pages 7-9, 13-16, 21-25 and table 2	1,3-6
X		1D,1I, 3D,3I
	--	
Y	WO, A1, 8602383 (INSTITUT PASTEUR ET AL.) 24 April 1986, see claim 9, pages 21-24 and 43-45	1,3-6
X		1D,3D
	--	

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
23rd May 1991	1991 -06- 03
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Carl Olof Gustafsson Carl Olof Gustafsson

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	WO, A1, 8705399 (CENTOCOR, INC.) 11 September 1987, see fig. 1 page 6 - page 7, line 17 and pages 13-19. See in particular page 18, lines 1-4 --	1,3-6
A	WO, A1, 8606099 (GENETICS SYSTEMS CORPORATION) 23 October 1986, see pages 9-10, 20-23, 30-38, see in particular page 32, lines 9-28 --	1,3-6
X	AIDS, Vol. 3, No. 12, 1989 R. Bridget Ferns et al.: "Epitope location of 13 anti-gag HIV-1 monoclonal antibodies using oligopeptides and their cross reactivity with HIV-2", see page 829 - page 834 see table 1 --	1B,D,3B, D
X	Journal of Virology, Vol. 63, No. 8, 1989 M. Niedrig et al.: "Epitope Mapping of Monoclonal Antibodies against Human Immunodeficiency Virus Type 1 Structural Proteins by Using Peptides", see page 3525 - page 3528 see Table 1 --	1D,3D
X	NATURE, Vol. 336, December 1988 Douglas F. Nixon et al.: "HIV-1 gag-specific cytotoxic T lymphocytes defined with recombinant vaccinia virus and synthetic peptides", see page 484 - page 487 see the whole document --	1F,3F
X	Immunology, Vol. 67, 1989 T. Mathiesen et al.: "Mapping of IgG subclass and T-cell epitopes on Hiv proteins by synthetic peptides", see page 453 - page 459 see Table 2, peptide 59 --	1F
X	Journal of Acquired Immune Deficiency Syndromes, Vol. 4, 1989 (New York) B. Wahren et al.: "HIV-1 Peptides Induce a Proliferative Response in Lymphocytes from Infected Persons", see page 448 - page 456 see table 2, peptide 59 and table 1, peptides 33-85 --	1F,3F

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	EP, A1, 0290893 (GENETIC SYSTEMS CORPORATION) 17 November 1988, see pages 5-6 --	1I,3I
X	GB, A, 2196634 (GENETIC SYSTEMS CORPORATION) 5 May 1988, see pages 6, 11, 13 and claim 35 --	1I,3I
P,X	WO, A1, 9007119 (IMMUNODIAGNOSTICS, INC.) 28 June 1990, see fig. 8 --	1I,3I

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V.☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1.☐ Claim numbers .... because they relate to subject matter not required to be searched by this Authority, namely:

2.☐ Claim numbers ...., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3.☐ Claim numbers.......... because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI.☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

See next sheet !

1.☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2.☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3.☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4.☒ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED

Lack of unity a priori.

The present application refers to the solutions of two different problems as listed below. No technical relationship can be appreciated to be present so as to form a general inventive concept.

A. Immunoassay for antibodies involved in false positive results in HIV tests and use of long p24 peptides as reagents according to claims 1a,c,e,g,h, 2, 3a,c,e,g,h and 4-6.

B. Short peptides and reagents (for use in the delineation of p24 epitopes) according to claims 1b,d,f,i and 3b,d,f,i.

Lack of unity a posteriori.

The general problem underlying the invention B above is not novel and most of the documents cited in the search report relate to the mapping of epitopes in the p24 region (cf. AIDS vol. 3, 1989, 829 ff and Immunology vol. 67, 1989, p. 453-459). This leads to the regrouping of B under distinct subjects as listed below, each subject now falling under its own inventive concept.

B1	Peptide	and	diagnostic	antigen	according	to	claims	1b and 3b
B2	"	"	"	"	"	"	"	1d and 3d
B3	"	"	"	"	"	"	"	1f and 3f
B4	"	"	"	"	"	"	"	1i and 3i

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.PCT/SE 91/00114

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the Swedish Patent Office EDP file on 91-04-30
The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A2- 0330359	89-08-30	AU-D-	3076189	89-08-31
		JP-A-	1308299	89-12-12
WO-A1- 8606414	86-11-06	AU-B-	571128	88-03-31
		AU-B-	597884	90-06-14
		AU-D-	5572786	86-10-16
		AU-D-	5773386	86-11-18
		EP-A-	0201716	86-11-20
		EP-A-	0220273	87-05-06
		JP-T-	62502617	87-10-08
		US-A-	4629783	86-12-16
		US-A-	4768607	88-09-06
WO-A1- 8602383	86-04-24	AU-D-	5061785	86-05-02
		EP-A-	0201540	86-11-20
		EP-A-	0387914	90-09-19
		EP-A-	0387915	90-09-19
		FR-A-B-	2571968	86-04-25
		JP-T-	62500592	87-03-12
		AU-B-	603543	90-11-22
		AU-B-	600227	90-08-09
		AU-D-	5320086	86-08-13
		EP-A-B-	0211022	87-02-25
		JP-T-	62502095	87-08-20
		OA-A-	8413	88-06-30
		WO-A-	86/04336	86-07-31
WO-A1- 8705399	87-09-11	EP-A-	0258404	88-03-09
		JP-T-	1500053	89-01-12
		US-A-	4808536	89-02-28
WO-A1- 8606099	86-10-23	AU-B-	571128	88-03-31
		AU-B-	594333	90-03-08
		AU-D-	5572786	86-10-16
		AU-D-	5669086	86-11-05
		EP-A-	0201716	86-11-20
		EP-A-	0220234	87-05-06
		JP-A-	62239992	87-10-20
		JP-T-	62502723	87-10-22
		US-A-	4784941	88-11-15
EP-A1- 0290893	88-11-17	AU-D-	1508088	88-11-03
		AU-D-	7720187	88-05-19
		BE-A-	1000811	89-04-11

ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.PCT/SE 91/00114

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the Swedish Patent Office EDP file on 91-04-30
The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A1- 0290893	88-11-17	CH-A-	675728	90-10-31
		DE-A-	3727703	88-05-05
		FR-A-	2603107	88-02-26
		GB-A-	2196634	88-05-05
		JP-A-	1063392	89-03-09
		JP-A-	1085928	89-03-30
		LU-A-	86972	88-03-02
		NL-A-	8701950	88-03-16
		OA-A-	8652	88-11-30
		SE-A-	8703225	88-02-21
GB-A- 2196634	88-05-05	AU-D-	1508088	88-11-03
		AU-D-	7720187	88-05-19
		BE-A-	1000811	89-04-11
		CH-A-	675728	90-10-31
		DE-A-	3727703	88-05-05
		EP-A-	0290893	88-11-17
		FR-A-	2603107	88-02-26
		JP-A-	1063392	89-03-09
		JP-A-	1085928	89-03-30
		LU-A-	86972	88-03-02
		NL-A-	8701950	88-03-16
		OA-A-	8652	88-11-30
		SE-A-	8703225	88-02-21
WO-A1- 9007119	90-06-28	AU-D-	4816690	90-07-10